

We claim:

1. A method of making an array of selected anti-ligands comprising:

- (i) providing a library of anti-ligand molecules displayed for binding with a ligand on the surface of a replicable unit;
- (ii) providing a mixture of ligands;
- (iii) exposing the library to the mixture whereby ligand/anti-ligand binding can take place;

(iv) isolating and amplifying the number of anti-ligands which bind ligands; and

(v) applying a preparation of the same anti-ligands, or a plurality of different anti-ligands, to a separate region of a substrate to form an array of separate anti-ligand-containing regions on a solid support.

2. A method as claimed in Claim 1, further comprising a step of isolating ligands bound to anti-ligands on the surface of the replicable units between steps (iii) and (iv).

3. A method as claimed in Claim 1 wherein the ligands in the mixture are immobilised.

4. A method as claimed in Claim 1 wherein the mixture of ligands is separated on the basis of one or more parameters before it is exposed to the library.

5. A method as claimed in Claim 4 wherein the mixture of ligands is separated using two-dimensional gel electrophoresis.

6. A method as claimed in Claim 5 wherein the ligands in the separated mixture are immobilised on a support surface.

7. A method as claimed in Claim 6 wherein the support surface is a nitrocellulose or polyvinylidene difluoride (PVDF) membrane.

8. A method as claimed in Claim 6 wherein the support surface is a replica of the two-dimensional gel and is used directly in step (iii) of the method of Claim 1.

9. A method as claimed in Claim 2 wherein the ligands in the mixture are tagged by a tagging agent so that they can be isolated by an anti-tagging agent which binds to the tagging agent.

10. A method as claimed in Claim 9 wherein the tagging agent is biotin and the anti-tagging agent is avidin.

11. A method as claimed in claim 1 wherein the anti-ligand comprises a protein or polypeptide.

12. A method as claimed in Claim 11 wherein the anti-ligand is an antibody or an antigen binding variant or derivative thereof.

13. A method as claimed in claim 1 wherein the anti-ligand is a nucleic acid.

14. A method as claimed in claim 1 wherein the identity of at least some of the ligands and/or anti-ligands is unknown.

15. A method as claimed in Claim 14 wherein the identity of substantially all of the ligands and/or anti-ligands is unknown.

16. A method as claimed in claim 1 wherein from 10 to 50 different anti-ligands are applied per region of the array.

17. Use of an array obtainable by a method as claimed in claim 1 in a method comprising comparing the presence, absence and/or amount of one or more ligands in first and second biological samples by detecting differences in ligand/anti-ligand binding when the array is exposed to the samples.

18. Use of two or more substantially identical arrays obtainable by a method as claimed in claim 1 in a method comprising comparing the presence, absence and/or amount of one or more ligands in first and second biological samples by detecting differences in ligand/anti-ligand binding when an array is exposed to the first biological sample and a substantially identical array is exposed to the second biological sample.

19. A use as claimed in claim 17 wherein the ligands in the first and second biological samples are labelled with different first and second fluorescent reporters so that, in use, under examination of the array under conditions of fluorescence excitation, anti-ligands in the array which are bound predominantly to ligands from one of the first and second biological samples give a first or second fluorescence emission; and anti-ligands which bind substantially equal numbers of ligands from the first and second biological samples give a combined fluorescence emission.

20. A use as claimed in claim 18 wherein the ligands in the first and second biological samples are labelled with different first and second fluorescent reporters so that, in use, under examination of the array under conditions of fluorescence excitation, anti-ligands in the array which are bound predominantly to ligands from one of the first and second biological samples give a first or second fluorescence emission; and anti-ligands which bind substantially equal numbers of ligands from the first and second biological samples give a combined fluorescence emission.

21. A use as claimed in Claim 17 wherein the first and second biological samples are applied to identical but separate arrays of anti-ligands.

22. A use as claimed in claim 18 wherein the first and second biological samples are applied to identical but separate arrays of anti-ligands.

23. A use as claimed in claim 17 wherein the mixture of ligands provided in step (ii) of the method of making the array is derived from the same source as the first or second biological sample.

24. A use as claimed in claim 18 wherein the mixture of ligands provided in step (ii) of the method of making the array is derived from the same source as the first or second biological sample.

25. A use as claimed in claim 17 wherein the first biological sample is from a diseased cell type and the second biological sample is from a corresponding cell type unaffected by the disease.

26. A use as claimed in claim 18 wherein the first biological sample is from a diseased cell type and the second biological sample is from a corresponding cell type unaffected by the disease.